

# Significance of ovomucoid- and ovalbumin-specific IgE/IgG<sub>4</sub> ratios in egg allergy

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**Background:** The role of specific IgG<sub>4</sub> antibodies in natural tolerance acquisition remains a matter of debate; the specific IgE/IgG<sub>4</sub> ratio might add value to the measurement of absolute amounts of IgE for assessing the ongoing status of egg reactivity. **Objective:** We sought to determine the significance of IgG<sub>4</sub> antibodies to ovalbumin (OVA) and ovomucoid (OVM) in egg-allergic children.

**Methods:** One hundred seven egg-allergic children (mean age 6.9 years; range 1.6–18.6 years) were challenged to baked egg. The outcomes of the challenges were related to the level of specific IgE and IgG<sub>4</sub> to OVM and OVA, component IgE/IgG<sub>4</sub> ratios, and mediator release in a functional assay based on the rat basophil leukemia cell line.

**Results:** Baked egg-reactive children had significantly higher OVA and OVM ratios of IgE/IgG<sub>4</sub> and mediator release in the rat basophil leukemia-based assay than did tolerant children ( $P < .05$  for both). The OVA- and OVM-specific IgE/IgG<sub>4</sub> ratios and mediator release were correlated. In the receiver operating characteristic analysis, the areas under the curve for a logistic regression model including specific IgE and IgG<sub>4</sub> to OVA and OVM were significantly greater compared with the areas under the curve for egg white-specific IgE and OVM-specific IgE.

**Conclusions:** The balance between IgE and IgG<sub>4</sub> to OVA and OVM has functional consequences. A model that includes the interactions between IgE and IgG<sub>4</sub> to OVA and OVM accurately predicts reactivity to baked egg and warrants further investigation. (J Allergy Clin Immunol 2012;■■■■:■■■■–■■■■.)

**Key words:** Egg, egg white, ovalbumin, ovomucoid, egg allergy, food allergy, children, hypersensitivity, IgE, IgG<sub>4</sub>, IgE/IgG<sub>4</sub> ratio

Hen's egg white allergy affects approximately 1.6% of children.<sup>1–4</sup> In a large ( $n = 2848$ ), population-based study from Australia, the prevalence of challenge-proven, uncooked-egg allergy in 12-month-old infants was 8.9% (95% CI, 7.8–10.0).<sup>5</sup> Egg allergy prevalence is considerably higher, up to 30%, in children with atopic dermatitis or other food allergies.<sup>6,7</sup> Although egg allergy usually resolves with age, it can persist beyond the age of 5 years.<sup>8,9</sup> The majority (>70%) of children reacting to regular egg (lightly heated such as French toast or scrambled egg) can tolerate ingestion of baked egg.<sup>5,10–15</sup> Consumption of baked egg (muffin and waffle) by children allergic to regular egg (French toast, scrambled egg) was associated with immunologic changes that suggested accelerated development of tolerance to regular egg.<sup>14</sup>

Although our knowledge of the pathophysiology of food allergy has improved, mechanisms of tolerance development remain poorly understood.<sup>16,17</sup> In type I food hypersensitivity, food-specific IgE antibodies are implicated in the pathophysiology. Emerging data suggest a role for the IgG<sub>4</sub> immunoglobulin class in tolerance development. Naturally developing tolerance to cow's milk was associated with increased regulatory T cells and increased casein-IgG<sub>4</sub> levels.<sup>18–20</sup> In oral immunotherapy trials, increasing food-specific IgG<sub>4</sub> levels in sensitized individuals accompanied acquisition of clinical tolerance.<sup>21,22</sup> Protective or blocking functions for IgG<sub>4</sub> subclass antibodies have been proposed<sup>23,24</sup>; in peanut, egg, and milk allergy, IgG<sub>4</sub> antibodies generally overlap with IgE antibodies in respect to sequential epitope specificity.<sup>25–28</sup>

We sought to determine the functional significance of IgG<sub>4</sub> antibodies to ovalbumin (OVA) and ovomucoid (OVM) in children with IgE-mediated egg allergy. We hypothesized that high IgE/IgG<sub>4</sub> ratios to OVA and OVM are associated with a higher likelihood of reactivity to baked egg in egg-allergic children. We evaluated the performance of a logistic regression model that includes both specific IgE and IgG<sub>4</sub> to OVA and OVM for predictions of reactivity to baked egg.

## METHODS

### Study population

We analyzed data from a study on tolerance to baked egg.<sup>14</sup> Tolerance to baked egg was determined by oral food challenge (OFC). The study was

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**Abbreviations used**

EW: Egg white  
OFC: Oral food challenge  
OVA: Ovalbumin  
OVM: Ovomucoid  
RBL: Rat basophil leukemia

approved by the Mount Sinai Institutional Review Board, and informed consent was obtained before enrollment.

**Antibody measurements**

A serum sample was collected from each subject to measure egg white (EW)-, OVM-, and OVA-specific IgE and OVM- and OVA-specific IgG<sub>4</sub> antibody concentrations with the UniCAP system (Phadia US, Portage, Mich). For specific IgE, the lower limit of detection was 0.35 kU<sub>A</sub>/L and the upper limit of detection was 100 kU<sub>A</sub>/L. For food-specific IgG<sub>4</sub>, the lower limit of detection was 0.02 mg<sub>A</sub>/L and the upper limit of detection was 35 mg<sub>A</sub>/L. For analysis, values below the lower limit of detection for specific IgG<sub>4</sub> and IgE were replaced by half the lower limit of detection,<sup>29</sup> that is, 0.01 mg<sub>A</sub>/L for IgG<sub>4</sub> and 0.175 kU<sub>A</sub>/L for IgE.

**Mediator release assay**

Sera were obtained from baked egg–reactive children ( $n = 10$ ), baked egg–tolerant/regular egg–reactive children ( $n = 9$ ), and children with a history of egg allergy who tolerated baked egg and regular egg during an OFC ( $n = 8$ ). Rat basophil leukemia (RBL) cells transfected with human Fcε receptor 1 (confirmed expression of alpha chain; kind gift from Dr Stefan Vieths and Dr Lothar Vogel) are an accepted *in vitro* model for studying IgE-mediated reactions.<sup>30</sup> RBL cells were passively sensitized with human serum overnight and stimulated with a range of 10-fold serial dilutions of unheated and heated (>98.5°C [203°F] for 30 minutes) EW powder, purified OVA grade V, and OVM grade VII (Sigma, St Louis, Mo), starting concentration of 32 μg/mL. The assay was performed as previously published.<sup>31</sup> N-Hexosaminidase was measured in the supernatant as a marker of RBL-cell mediator release. Results were expressed as a percentage of the cell release minus the spontaneous release that was divided by the total release.

**Statistical analysis**

Analyses were performed by using SAS/STAT version 9.2 (SAS Institute, Inc, Cary, NC). Comparison of data between baked egg–reactive and baked egg–tolerant patients was performed with the Mann-Whitney *U* test. A probability level of less than 5% was considered as significant. The Spearman rank order correlation coefficient ( $r_s$ ) was used to measure the strength of the relationship between specific IgE and specific IgG<sub>4</sub>. A Wilcoxon rank-sum test was used to compare peak mediator release in baked egg–reactive and baked egg–tolerant subjects. The linear relationship between peak mediator release and specific IgE/IgG<sub>4</sub> ratios to OVA and OVM was assessed with a mixed model, which accounted for the correlation between repeated measures made on each subject.

A logistic regression model was used to estimate the probability of baked egg allergy among different combinations of specific IgE and IgG<sub>4</sub> to OVA and OVM by using the following formula:  $1/(1 + \exp(-2.33 + 0.26(\text{specific IgE to OVA}) + 0.19(\text{specific IgE to OVM}) - 0.18(\text{specific IgG}_4 \text{ to OVA}) + 0.99(\text{specific IgG}_4 \text{ to OVM}) - 0.12(\text{specific IgE to OVA} \times \text{specific IgG}_4 \text{ to OVA}) - 0.34(\text{specific IgE to OVM} \times \text{specific IgG}_4 \text{ to OVM})))$ . Receiver operating characteristic curves were generated for specific IgE to EW, OVM, and OVA as well as for the logistic regression model. The areas under the curves were estimated and compared by using PROC LOGISTIC in SAS. Youden's index, the value that maximizes the true-positive rate while minimizing the false-positive rate,<sup>32</sup> was used to estimate the optimal cutpoint (dichotomizing the predicted probabilities of response) for each curve. Using the

dichotomized version of these predicted probabilities of baked egg allergy, screening diagnostic statistics including sensitivity, specificity, positive predictive value, and negative predictive value were computed along with 95% CIs.

**RESULTS****Baseline clinical characteristics**

One hundred seventeen children, mean age of 6.9 years (range, 1.6–18.6 years), were enrolled; 10 were excluded from the analysis because of missing specific IgE and IgG<sub>4</sub> data (Table I). Twenty-five subjects (group A) reacted to baked egg when challenged. They were considered allergic to both baked and regular egg. Of the 82 subjects who tolerated baked egg, 35 reacted to regular egg during the OFC. Twenty-one subjects were not challenged to regular egg, because of EW-specific IgE or skin prick test values greater than the highly predictive levels of reactivity or because of a recent (within previous 6 months) convincing clinical reaction to regular egg. Four subjects refused the regular egg challenge. These 60 patients were grouped together (group B). The remaining 22 subjects were tolerant to both baked and regular egg (group C).

**Baseline immunologic parameters in relation to the outcome of the baked egg OFC**

The levels of specific IgE to EW, OVM, and OVA measured at baseline during strict avoidance of dietary egg were significantly higher in baked egg–reactive subjects (group A) than in baked egg–tolerant subjects (groups B and C) ( $P < .05$ ) (Table I). There was a strong correlation between IgE antibody levels to OVA and to EW ( $r_s = 0.95$ ;  $P < .001$ ), between specific IgE to OVM and to EW ( $r_s = 0.8$ ;  $P < .001$ ), and between specific IgE to OVA and to OVM ( $r_s = 0.77$ ;  $P < .001$ ). Most subjects had detectable IgE to both OVM and OVA; 5 subjects (20%) reacting to baked egg had high levels of IgE to OVA and undetectable IgE to OVM.

As previously reported,<sup>14</sup> OVA- and OVM-specific IgG<sub>4</sub> levels did not differ significantly between subjects reactive and tolerant to baked egg ( $P = .78$  and  $P = .77$ , respectively). The correlation between specific IgE and specific IgG<sub>4</sub> was modest, although significant for OVA ( $r_s = 0.33$ ;  $P < .001$ ) and OVM ( $r_s = 0.53$ ;  $P < .001$ ).

A subgroup of subjects was found to have undetectable IgG<sub>4</sub> to OVM and/or to OVA. Nine of the 23 subjects (39.1%) with undetectable IgG<sub>4</sub> to both OVM and OVA never knowingly ate egg before the OFC, whereas only 8 subjects out of the 84 (9.5%) with detectable specific IgG<sub>4</sub> to OVA and/or to OVM had never knowingly eaten egg before ( $P = .002$ ). The majority of patients (55 of 62; 88.7%) who reported a reaction to egg in the past had positive specific IgG<sub>4</sub> to OVM and/or OVA.

**OVA- and OVM-specific IgE/IgG4 ratios in different groups of subjects**

OVA- and OVM-specific IgE/IgG<sub>4</sub> ratios were significantly higher in baked egg–reactive subjects (group A) than in baked egg–tolerant subjects (groups B and C) ( $P = .001$  and  $P = .003$ , respectively) (Fig 1). Of note, differences in OVA- and OVM-specific ratio between groups remain significant after adjusting for specific IgE to OVA and OVM by analysis of covariance using ranks ( $P < .03$ ). The correlation coefficient between the IgE/IgG<sub>4</sub> ratio to OVA and to OVM was high ( $r_s = 0.73$ ;  $P < .001$ ), as well as

TABLE I. Study participants' characteristics

Outcome of the oral challenge	Group A Baked egg reactive	Group B Baked egg tolerant*	Group C Regular egg tolerant†
No. of patients, n (%)	25 (23.4)	60 (56.1)	22 (20.5)
Age (y)			
Mean	7.2	6.5	7.9
Range	1.8-13.1	1.6-15.8	3.4-18.6
Gender, n (%)			
Male	21 (84)	38 (63.3)	13 (59.1)
Female	4 (16)	22 (36.7)	9 (40.9)
Severity of the reaction during OFC,‡ n (%)			
Grade 1	8 (32)	12 (34.3)	0
Grade 2	6 (24)	11 (31.4)	0
Grade 3	5 (20)	4 (11.4)	0
Grade 4	6 (24)	8 (22.9)	0
Grade 5	0 (0)	0 (0)	0
Specific IgE (kU <sub>A</sub> /L), mean (range)			
EW	11.1 (0.7-58.9)	6.1 (0.2-83.6)	1.9 (0.2-9.4)
OVA	11.9 (0.8-61.8)	6.2 (0.2-86.1)	1.6 (0.2-9.7)
OVM	8.9 (0.2-51.8)	2.8 (0.2-32.6)	1.8 (0.2-20.1)
Specific IgG <sub>4</sub> (mg <sub>A</sub> /L), mean (range)			
OVA	0.4 (0.0-2.9)	1.5 (0.0-11.8)	2.4 (0.0-31)
OVM	0.2 (0.0-1.5)	0.4 (0.0-5.4)	0.8 (0.0-14.1)

To be noted, all patients included in this study were on strict avoidance of dietary egg before the challenge to determine tolerance to baked and/or uncooked egg.

ND, Nondetermined.

\*Baked egg tolerant (including 35 patients tolerating baked egg but reacting to regular egg and 25 patients tolerating baked but not challenge to regular egg).

†Regular egg tolerant; children with diagnosis of egg allergy who were asymptomatic during baked egg and regular egg challenge.

‡Grading according to Sampson et al.<sup>53</sup>

the correlation between specific IgG<sub>4</sub> to OVM and to OVA ( $r_s = 0.76$ ;  $P < .001$ ). Baked egg-reactive patients with levels of specific IgE of more than 7 kU<sub>A</sub>/L to EW had a significantly higher IgE/IgG<sub>4</sub> ratio compared with tolerant patients with similar levels of specific IgE to EW ( $P = .03$ ). The majority of children with more severe reactions during the OFC who were treated with epinephrine had a high IgE/IgG<sub>4</sub> ratio to OVM and/or to OVA (Table II). Most of these patients (9 of 14; 64.3%) also had a high value ( $>0.18$ , optimal cutoff decision point) from the statistical model. Children treated with epinephrine during the regular egg challenge had a significantly higher value (mean [ $\pm$ SEM] of  $0.26 [\pm 0.08]$ ) from the statistical model compared with patients not treated with epinephrine (mean [ $\pm$ SEM] of  $0.12 [\pm 0.01]$ ) ( $P = .02$ ). No difference was found among patients reacting to baked egg.

### Mediator release assay

Baked egg-reactive children had greater peak mediator release to unheated EW, OVM, and OVA than did tolerant children ( $P < .03$ ) (Fig 2). Heating (98.5°C [203°F] for 30 minutes) did not consistently decrease the mediator release caused by EW, OVA, and OVM. Heating decreased mediator release when cells were sensitized with serum from baked egg-tolerant children and stimulated

with EW powder. Heating of OVA and OVM did not decrease release.

The subjects with higher natural log transformed IgE/IgG<sub>4</sub> ratio to OVA were more likely to have nonzero mediator release across a wide range of allergen concentrations ( $32-32^{-10}$   $\mu$ g/mL) ( $P = .02$ ), even after adjusting for specific IgE to OVA ( $P = .04$ ). Among subjects with nonzero mediator release, the natural log transformed IgE/IgG<sub>4</sub> ratio to OVA was positively associated with log normal mediator release (Fig 3, A), even after adjusting for specific IgE to OVA ( $P = .04$ ). Specifically, per 10% increase in the IgE/IgG<sub>4</sub> ratio to OVA, the mediator release increased by 2%. There was a similar relationship observed with the IgE/IgG<sub>4</sub> ratio to OVM; however, it did not reach statistical significance (Fig 3, B).

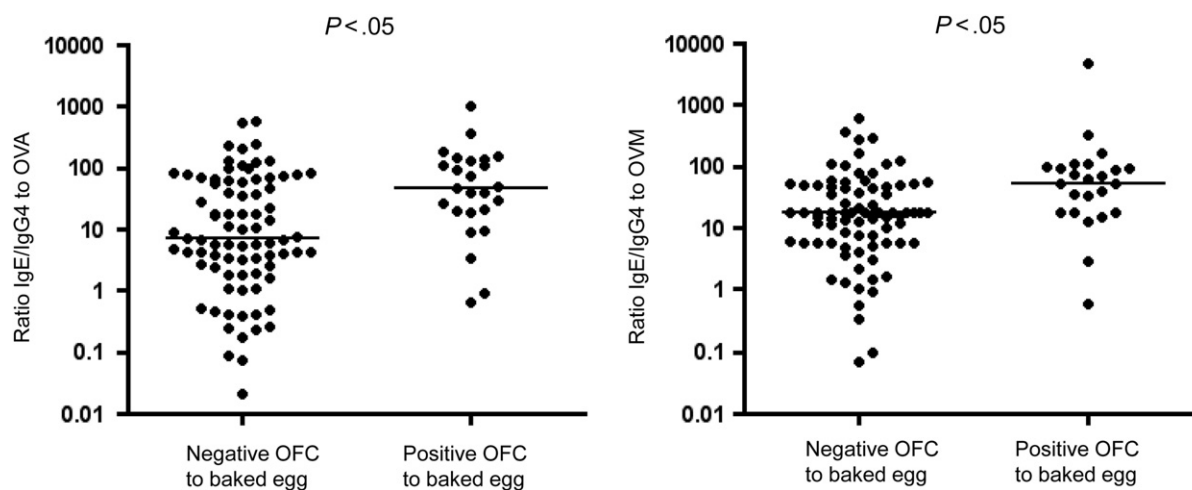
### Performance of different tests in prediction of tolerance to baked egg

The outcome of the OFC was considered as the reference parameter. The performance of the different tests was evaluated by receiver operating characteristic analysis. Baked egg-reactive subjects (group A) were compared with baked egg-tolerant subjects (groups B and C). In addition to specific IgE to EW, OVA, and OVM and specific IgG<sub>4</sub> to OVA and OVM, we evaluated a logistic regression model that incorporated specific IgE and IgG<sub>4</sub> to OVA and OVM. In our study population, the area under the curve for this model was significantly greater compared with the area under the curve for EW-specific IgE and OVM-specific IgE ( $P < .01$ ) (Fig 4). The model was also superior to OVA-specific IgE, but the difference did not reach statistical significance ( $P = .09$ ). We also found that the combination of specific IgE and IgG<sub>4</sub> to OVA or OVM in a logistic regression model significantly increased the area under the curve compared with specific IgE alone to OVA or OVM, respectively ( $P < .05$ ). The different decision points and the resulting clinical sensitivity and specificity for specific IgE to OVM and to EW as well as for the model including all the parameters are summarized in Table III.

### DISCUSSION

We report data in support of the hypothesis that specific IgG<sub>4</sub> antibodies play an important role in natural tolerance acquisition in egg-allergic children. Baked egg-reactive children have significantly higher OVA- and OVM-specific IgE/IgG<sub>4</sub> ratios than do tolerant children ( $P < .05$ ). By using logistic regression models, we showed that combination of specific IgE and IgG<sub>4</sub> performed better than specific IgE alone in predicting reactivity to baked egg.

The role of IgG<sub>4</sub> in tolerance acquisition has been debated for many years. Previous studies showed that IgG<sub>4</sub> levels increase during allergen-specific immunotherapy, indicating that IgG<sub>4</sub> antibodies play a role in the induction of tolerance.<sup>21,33</sup> IgG<sub>4</sub> antibodies have been proposed to act as blocking antibodies.<sup>24,34</sup> However, the role of IgG<sub>4</sub> antibodies in natural tolerance development in food allergy is poorly understood. So far, IgG<sub>4</sub> in cow's milk allergy has been studied, but the results are inconclusive.<sup>19,22</sup> Although it has been shown that attaining natural tolerance could be associated with higher IgG<sub>4</sub> levels<sup>19</sup> or an increase in sequential epitope binding by IgG<sub>4</sub>,<sup>26</sup> others reported higher IgG<sub>4</sub> levels in subjects allergic to cow's milk than in subjects without food allergy.<sup>22</sup> Regarding egg allergy, subjects



**FIG 1.** Results of the IgE/IgG4 ratio to OVA and OVM: comparison between children tolerating baked egg and those reacting to baked egg but tolerating regular egg.

**TABLE II.** Characteristics of children treated with epinephrine during OFC

Patient no.	Age (y)	Sex	Challenge food	Severity of reaction*	sIgE EW (kU <sub>A</sub> /L)	sIgE OVA (kU <sub>A</sub> /L)	sIgE OVM (kU <sub>A</sub> /L)	IgE/IgG <sub>4</sub> ratio for OVA	IgE/IgG <sub>4</sub> ratio for OVM	Statistical model†
1	9.3	M	Baked egg‡	Grade 4	5.9	6.9	6.6	49.9	165.3	0.99
2	4.9	M	Baked egg	Grade 4	8.8	11.7	13	19.8	100	0.86
3	1.9	M	Baked egg	Grade 4	58.9	61.8	51.8	92.2	63.1	0.64
4	7.7	M	Baked egg	Grade 4	7.7	9.9	0.7	39.8	74	0.52
5	8.9	M	Baked egg	Grade 4	3.3	3.3	3.3	46.3	40.9	0.29
6	8.8	F	Baked egg	Grade 4	0.9	1.8	0.5	183	54	0.15
7	6.9	F	Regular egg§	Grade 4	4.3	4.9	5.9	122.8	296	0.51
8	6.9	F	Regular egg	Grade 4	1.02	1.9	1.6	63.6	161	0.18
9	3.5	M	Regular egg	Grade 4	0.6	0.6	0.5	59	48	0.11
10	4.2	M	Regular egg	Grade 4	2.5	0.6	1.9	1.1	5.6	0.14
11	5.9	M	Regular egg	Grade 4	6.7	9.3	9.4	46.6	23.5	0.69
12	8.3	M	Regular egg	Grade 4	0.5	0.6	0.6	57	56	0.11
13	7.8	F	Regular egg	Grades 2-3	2.2	2.03	1.8	2.7	5.7	0.16
14	13.8	F	Regular egg	Grades 2-3	4.3	2.9	1.04	6.6	4.95	0.19

sIgE, Specific IgE.

\*Grading according to Sampson et al.<sup>53</sup>

†Statistical model (logistic regression) including both specific IgE and IgG<sub>4</sub> to OVA and OVM; optimal cutoff decision point for this model: 0.18.

‡Baked egg; muffin and a waffle.

§Regular egg; French toast or scrambled egg.

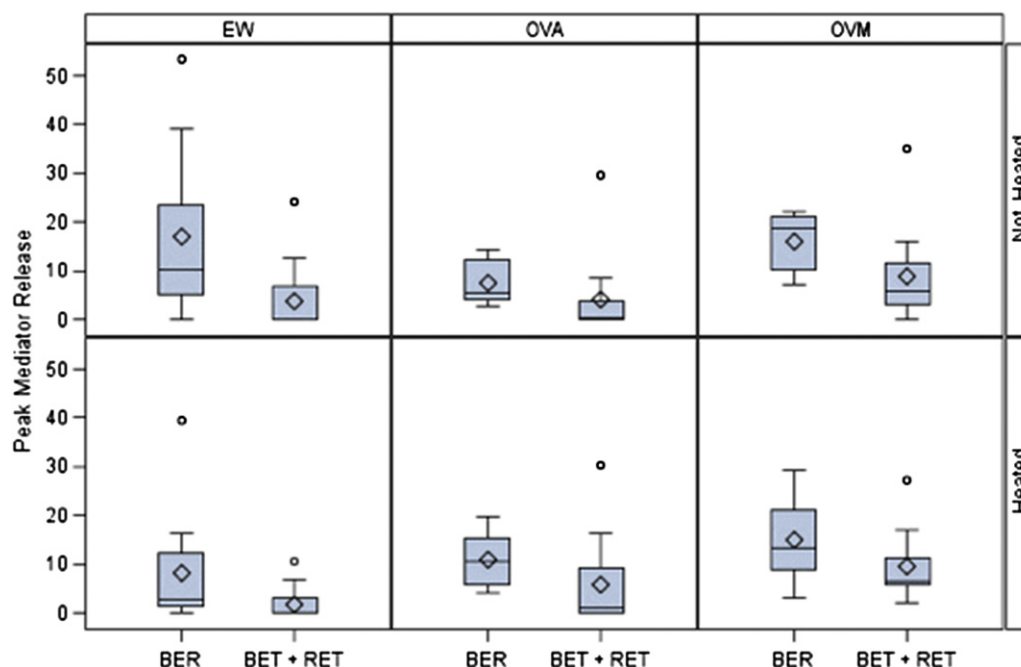
with persistent sensitization and allergy have increased OVA-specific IgG levels compared with those with transient sensitization to EW.<sup>35,36</sup> Confirming previous reports,<sup>37</sup> we found that the levels of OVA- and OVM-specific IgG<sub>4</sub> did not correlate with the reactivity to baked egg ( $P = .78$  and  $P = .77$ , respectively). As the absolute numbers of specific IgG<sub>4</sub> levels do not seem to be predictive of tolerance, measurement of specific IgG<sub>4</sub> level alone is not helpful in the diagnostic evaluation of suspected egg allergy and this test alone should not be used in clinical practice.<sup>4,38</sup>

IgG production is a physiological response to ingested dietary antigens, which can be detected in a large proportion of tolerant subjects.<sup>39-43</sup> In our study, previous exposure to egg was reported in all subjects with positive IgG<sub>4</sub> to OVA and/or OVM. We found that 9 subjects (39.1%) with undetectable specific IgG<sub>4</sub> to both OVA and OVM had never been knowingly exposed to egg. These

subjects had detectable specific IgE to OVA and/or OVM. Our results are in agreement with findings in beekeepers,<sup>44</sup> suggesting that it takes many months of repeated antigen exposure before IgG<sub>4</sub> responses become prominent. Production of sufficient IL-10 is likely a rate-limiting step.<sup>44</sup>

The balance between allergen-specific IgE and IgG<sub>4</sub> may influence whether clinical allergy persists or tolerance develops.<sup>18,19,45,46</sup> The production of IgE and IgG<sub>4</sub> antibodies is regulated by similar mechanisms; for example, IL-4 from T<sub>H</sub>2 cells induces both IgE and IgG<sub>4</sub> switching in B cells, whereas IL-10 inhibits IgE secretion but upregulates IgG<sub>4</sub> secretion.<sup>34,47</sup> The IgE/IgG<sub>4</sub> ratio has been proposed as a measure of the specific-IL-10-driven humoral response. In studies of allergen immunotherapy, immunological tolerance occurred through a modified T<sub>H</sub>2 response characterized by high IgG<sub>4</sub>/IgE ratio.<sup>48-50</sup> A recent study reported that food-sensitized infants





**FIG 2.** Mediator release caused by stimulation with unheated and heated EW, OVA, and OVM. *BER*, Baked egg-reactive children; *BET*, baked egg-tolerant children, but reacting to regular egg; *RET*, regular egg-tolerant children. Horizontal lines inside the box represent the median; diamonds in the box represent the mean; circles outside the box represent outlying observations more than 2.5 interquartile range from the 1st or 3rd quartiles.

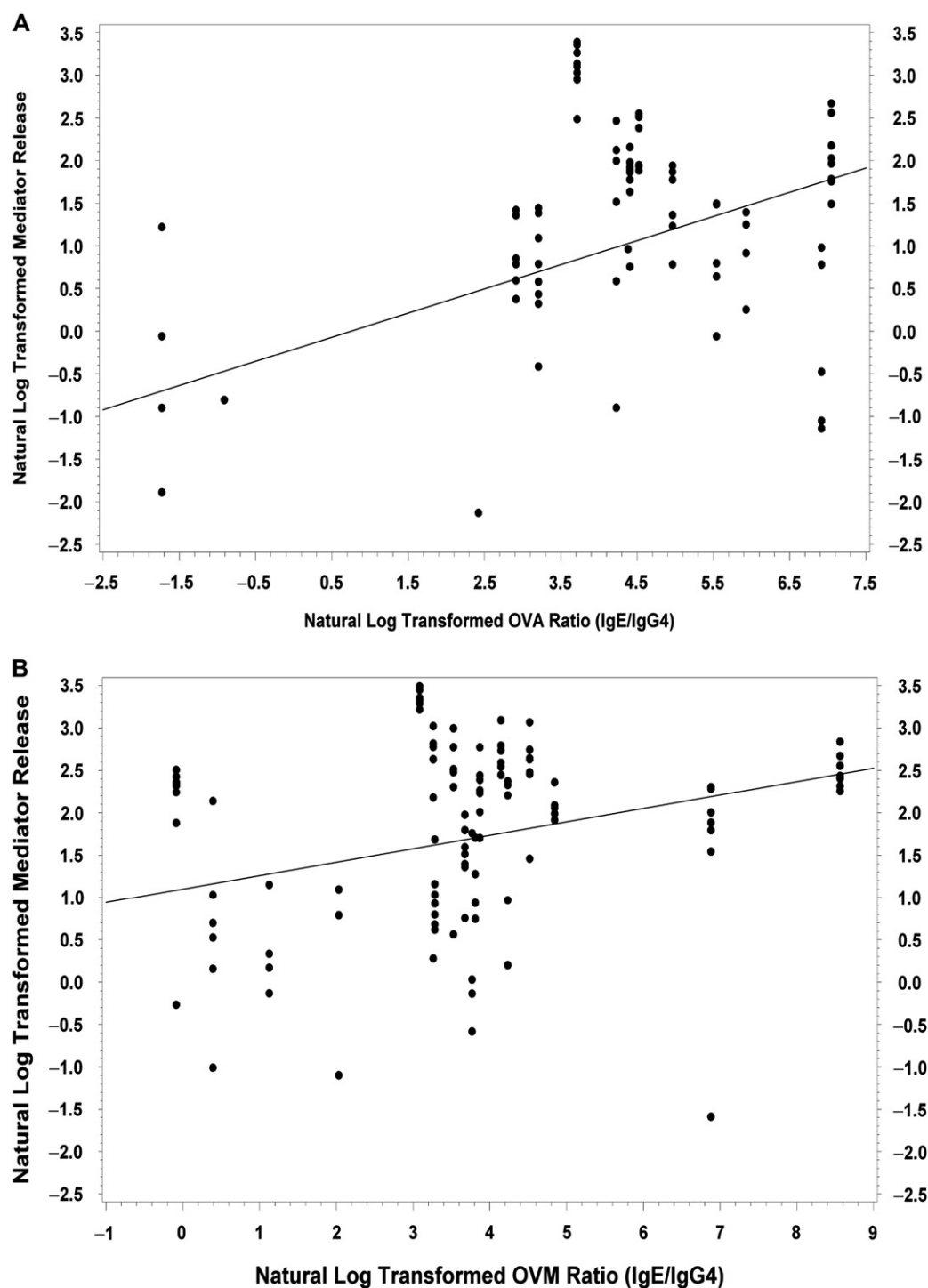
with high IgG<sub>4</sub>/IgE ratio to OVA were more likely to tolerate egg at 4 years than were infants with low ratios.<sup>46</sup> In the literature, both IgE/IgG<sub>4</sub> and IgG<sub>4</sub>/IgE ratios are reported. For practical reasons, we chose to report the ratios as IgE/IgG<sub>4</sub>. Our data showed that baked egg-reactive children had significantly higher IgE/IgG<sub>4</sub> ratios to OVA and OVM than did baked egg-tolerant children ( $P < .05$ ) (Fig 1). This increased IgE/IgG<sub>4</sub> ratio in baked egg-allergic children could be considered a marker of T<sub>H</sub>2 immune skewing as it correlates with allergy persistence. Importantly, the differences in IgE/IgG<sub>4</sub> ratios to OVA and OVM between groups persist after adjusting for specific IgE to OVA and OVM (analysis of covariance), confirming that our results were not being driven by only specific IgE itself. Moreover, the combination of IgE and IgG<sub>4</sub> could provide additional information to that provided by specific IgE alone. Particularly, the majority of children presenting with a severe reaction during the OFC had a high IgE/IgG<sub>4</sub> ratio to OVA and/or OVM, even those with a low level of specific IgE to EW (Table II).

We found a good correlation between the allergic status of the patients and mediator release (Fig 2) in an *in vitro* assay based on the RBL cell line. Heating of EW powder for 30 minutes at 98.5°C decreased mediator release when cells were sensitized with serum from baked egg-tolerant children. However, heating of OVA and OVM had no effect on mediator release, probably because 98.5°C is not a sufficiently high temperature to bring about the conformational changes seen with the high temperatures used in baking. The value of the OVA- and OVM-specific IgE/IgG<sub>4</sub> ratio and mediator release was correlated (Fig 3). IgG could block mast cell activation via inhibitory, Fcγ RIIB-dependent signals and via direct competition with IgE for allergenic epitopes.<sup>24</sup> Although cross-linking of IgG through the Fc receptor on RBL-2H3 cells

has been shown not to induce any detectable mediator release, RBL-2H3 cells express the Fcγ receptor.<sup>51</sup> Furthermore, murine Fcγ receptors recognize human IgG.<sup>52</sup> Therefore, the blocking effect of specific IgG<sub>4</sub> in our experiment could be explained by these 2 mechanisms. Alternatively, similar to the findings in children outgrowing milk allergy, the affinity of IgE antibodies in baked egg-tolerant children might be lower than in baked egg-reactive children, despite comparable IgE levels.<sup>26</sup>

Ando et al recently reported that OVM-specific IgE levels greater than 11 kU<sub>A</sub>/L were predictive of baked egg reactivity.<sup>12</sup> We found similar results with a positive decision point (corresponding to a specificity of 95%) for an OVM-specific IgE level of 12.8 kU<sub>A</sub>/L. However, in our study, specific IgE to EW and to OVA performed better than specific IgE to OVM (Fig 4). OVM is considered to be the most relevant allergen in EW, presumably because of its ability to maintain allergenicity despite extensive heating and acid treatment.<sup>27</sup> Our data support this hypothesis, showing significantly higher OVM-specific IgE levels in baked egg-reactive subjects than in baked egg-tolerant subjects. However, we found that 5 subjects reacting to baked egg had negative OVM-specific IgE but positive OVA-specific IgE. Moreover, among baked egg-tolerant but regular egg-reactive subjects, some had IgG<sub>4</sub> only to OVA (negative OVM-specific IgG<sub>4</sub>), despite having positive specific IgE to both OVA and OVM. Assuming that specific IgG<sub>4</sub> antibodies are markers of food tolerance, these subjects might have developed only IgG<sub>4</sub> to the relevant allergen, OVA. Our results support the concept that a subgroup of egg allergic children has specific reactivity to OVA without reactivity to OVM.

We developed a logistic regression model that incorporated both specific IgE and IgG<sub>4</sub> to OVA and OVM. Compared with

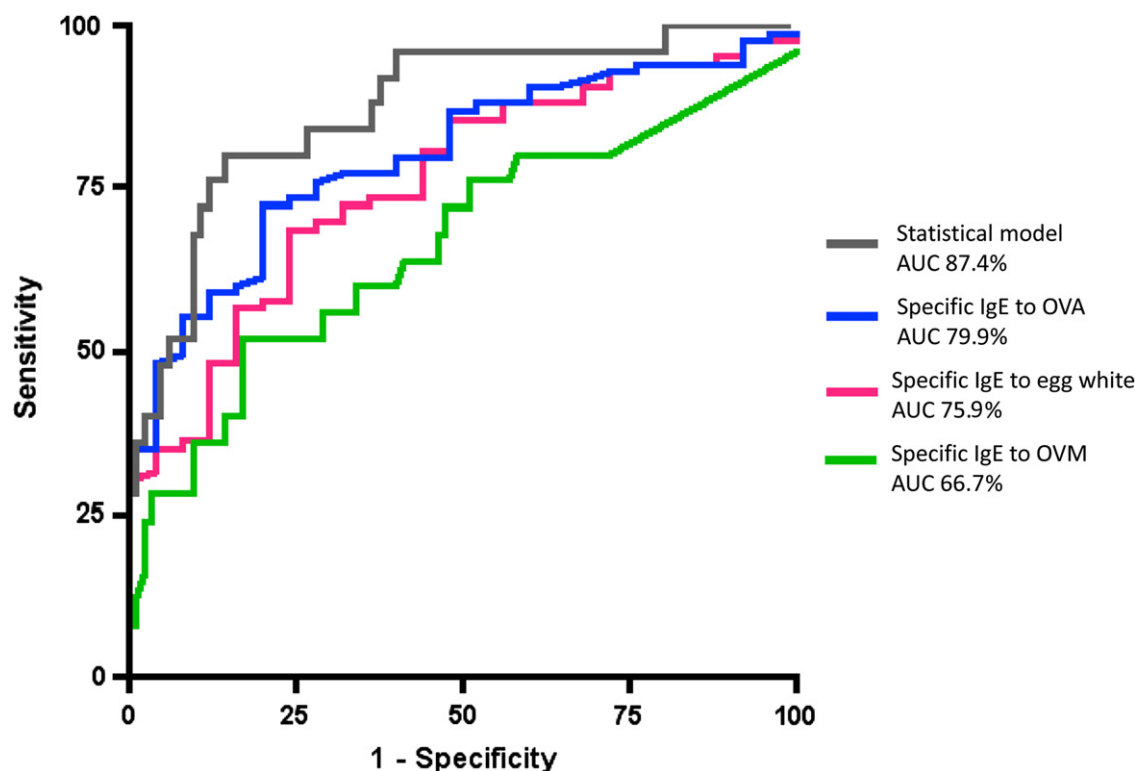


**FIG 3.** Correlation of the mediator release after stimulation with OVA (A) and OVM (B) and the IgE/IgG4 ratio to OVA and OVM, respectively.

specific IgE to EW or OVM, this model had the highest positive and negative predictive values (Table III) and was significantly better at differentiating between subjects reactive or tolerant to baked egg (Fig 4). By using the optimal cutoff decision point derived from the model, we found that a low value ( $<0.18$ ) from the statistical model was highly predictive of tolerance to baked egg, particularly in patients with levels of specific IgE to EW above 7 kU<sub>A</sub>/L (Fig 5). These data support a role for specific IgG<sub>4</sub> in

tolerance acquisition as analysis of the interaction between specific IgE and IgG<sub>4</sub> to OVA and/or OVM, rather than individual analysis, significantly improved the accuracy of diagnosing reactivity to baked egg.

There are several limitations inherent to our study design. First, exposure to egg is difficult to evaluate accurately, as children may have been exposed without the knowledge of their parents. Moreover, because the level of specific IgG may depend on the



**FIG 4.** Receiver operating characteristic curves showing the performance of the ImmunoCAP tests for specific IgE to EW, OVM, and OVA, as well as the statistical model, in relation to the diagnosis of children allergic to baked egg (ie, group A vs groups B and C). *AUC*, Area under the curve.

**TABLE III.** Characteristics of diagnostic tests for determination of clinical reactivity to baked egg

Diagnostic test	slgE to EW	slgE to OVM	slgE to OVA	Statistical model*
Area under the curve (%)	75.9	66.7	79.9	87.4
Optimal cutoff point†	2.6	3.3	2.67	0.18
Sensitivity, specificity (%)	76, 70	52, 83	80, 73	80, 85
PPV, NPV (%)	43, 91	48, 85	48, 92	63, 93
Positive decision point‡	26.2	12.8	25.3	0.52
Sensitivity, specificity (%)	12, 95	28, 95	8, 95	48, 95
PPV, NPV (%)	43, 78	64, 81	33, 77	75, 86
Negative decision point§	0.78	ND	1.29	0.12
Sensitivity, specificity (%)	96, 35		96, 49	96, 60
PPV, NPV (%)	31, 97		36, 98	42, 98

ND, Nondetermined (because the highest sensitivity is 80%); NPV, negative predictive value; PPV, positive predictive value; slgE, specific IgE.

\*Statistical model including both specific IgE and IgG<sub>4</sub> to OVM and OVA.

†Optimal cutoff point defined as the shortest distance to the receiver operating characteristic.

‡Positive decision point defined as the cutoff level producing a specificity of 95%.

§Negative decision point defined as the cutoff level producing a sensitivity of 95%.

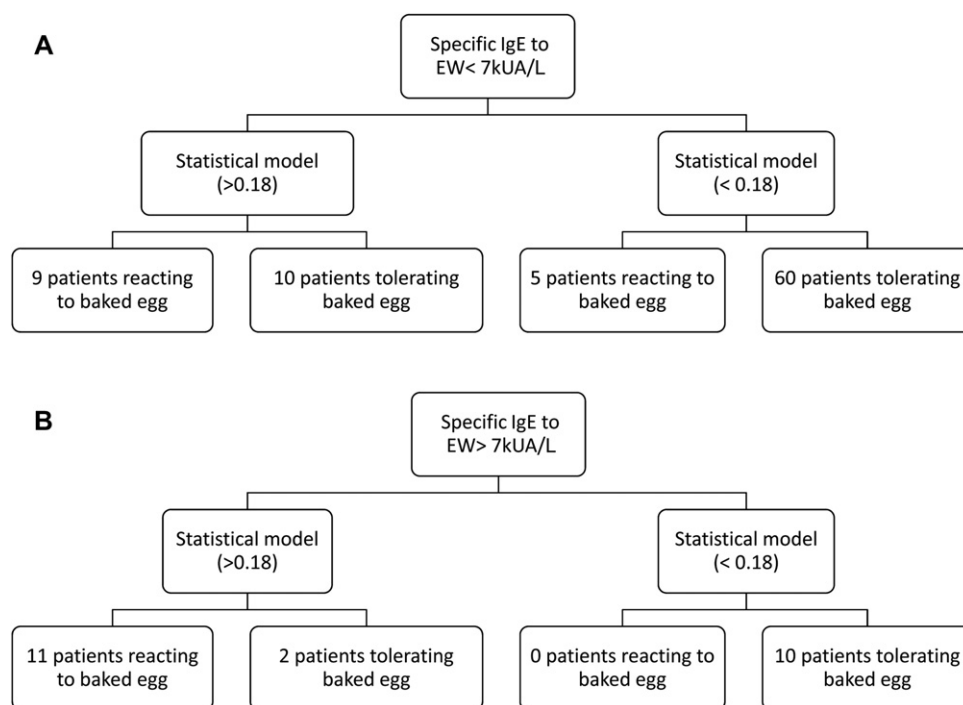
exposure level to egg, it is difficult to interpret these results. Recent studies showed that food-specific IgG levels were not solely related to dietary exposure,<sup>37,43</sup> but could reflect the polyisotypic response to allergen. Food-allergic subjects produced significantly more food-specific antibodies than did controls despite the fact that they remained on an allergen-free diet. However, none of these studies included systematic dietary reports with an objective evaluation of food consumption.

## Conclusions

Baked egg is a common ingredient in processed foods that is difficult to avoid in children's diets. Therefore, characterization of different phenotypes of egg allergy in relation to baked egg reactivity/tolerance is of practical significance. Our data suggest that the balance between IgE and IgG<sub>4</sub> to OVA and OVM has important functional implications. Children reactive to baked egg have high IgE/IgG<sub>4</sub> ratios to OVA and OVM, whereas children tolerant to baked egg have low IgE/IgG<sub>4</sub> ratios to OVA and OVM. High IgE/IgG<sub>4</sub> ratios to OVA and OVM are also associated with anaphylactic reactions requiring treatment with epinephrine during the OFC to both baked and regular egg. A logistic regression model that includes the interactions between specific IgE and IgG<sub>4</sub> to OVA and OVM has superior accuracy for predicting baked egg reactivity. These data support a protective role of specific IgG<sub>4</sub> in natural tolerance acquisition in food allergic subjects. From a diagnostic point of view, neither the IgE/IgG<sub>4</sub> ratio nor the statistical model is ready to be used in clinical practice and should be explored in future studies to evaluate their potential diagnostic value.

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**Clinical implications:** A low IgE/IgG<sub>4</sub> ratio to ovalbumin and ovomucoid is a marker of tolerance to baked egg in children. A statistical model that includes the interactions between specific IgE and IgG<sub>4</sub> to ovalbumin and ovomucoid predicts baked egg reactivity and warrants further investigation.



**FIG 5.** Statistical model including both specific IgE and IgG<sub>4</sub> to OVM and OVA as a diagnostic tool of baked egg allergy, using the optimal cutoff point in different groups of subjects. **A**, Subjects with low level of specific IgE to egg (<7 kUA/L), challenged to baked egg. **B**, Patients with high level of specific IgE to egg (>7 kUA/L), challenged to baked egg.

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